RELATIONS OF SOIL OLIGOCHAETES AND ALGAE

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The interaction was studied of some soil oligochaetes, earthworms, and enchytraeids, and soil algae. The use of labeled nitrogen showed the assimilation of algal biomass by the invertebrates studied. The selective assimilation of various algal species was suggested. The effect of the excrement of certain invertebrates on soil algae was studied. Analysis of the growth on earthworm coproliths showed that coproliths on the soil surface serve as a location for the multiplication of soil algae.

Previous investigations (Shtina et al., 1974; Kozlovskaya et al., 1975; Nekrasova and Artem'eva, 1978; Shtina and Nekrasova, 1971; Nekrasova et al., 1976) demonstrated that soil invertebrates play a major role in the population dynamics of algae and in the transformation of their biomass in soil. Algophages (or, more accurately, phycophages) are found at present in different systematic groups of animals, which belong, according to Gilyarov and Striganov (1978), to different size groups: nanofauna (many amoebas and ciliates), microfauna (tyroglyphid and oribatid ticks, springtails, rotifers, nematodes), and mesofauna (diplopods, insect larvae, earthworms, and enchytraeids).

Because of nutritional aspects, certain invertebrate groups eat algae in pure form (e.g., protozoa, ticks, springtails, fly larvae). Such instances have often been observed in nature and under cultivation conditions, and data on the amount of algae consumed by invertebrates have been obtained for these (Nekrasova and Domracheva, 1972; Domracheva, 1973; Nekrasova et al., 1976). Other animals, for example, lumbricids, enchytraeids, and diplopods, swallow algae together with soil or leaf litter. Here, the role of algae in nutrition was indicated either by a decrease in soil algal abundance in the presence of earthworms (Atlavinite and Pociene, 1973; Atlavinite, 1975) or by the drastic decrease in algal population in the intestines of animals in comparison with the starting nutritional material. Experiments, carried out according to the procedure of L. S. Kozlovskaya (Kozlovskaya et al., 1975; Nekrasova et al., 1976), revealed changes in the amount of algae added to the nutritional material (peat or leaf litter) during the passage of the material through the intestines of the earthworms, *Lumbricus terrestris* Hoff. and *Octolasium lacteum*, and the diplopod *Sarmatiulus kessleri*. In diplopods the number of algae in the excrement was two-threefold lower than in food, and in earthworms the number of algae in the lower portion of the intestine decreased 10-fold in comparison with the starting level. These experiments also revealed the selectivity of enchytraeid nutrition with respect to different algal species.

It was also demonstrated that some of the algae survive the passage through invertebrate intestines and that the invertebrates facilitate the distribution of algae in soil (Kozlovskaya et al., 1975). On the other hand, supported by observations of the intestinal content in earthworms and by counts of algae in soil inhabited by worms, O. P. Atlavinite (1975) concluded that all algae found in soil are totally digested in the worm intestine.

Thus, it was unclear to what extent the organic matter of algae is assimilated by invertebrates who pass soil or plant litter through their intestine, where algae are found in a distributed state. To solve this problem, we used in our research the isotope method, which makes it possible to determine assimilation of algae by invertebrates from the ^{15}N label.

Two groups of oligochaetes, earthworms and enchytraeids, were fed algae labeled with ^{15}N. Emission spectroanalysis of nitrogen isotope composition was carried out by O. S. Smetanin in the Department of Botany at the Kirov Agricultural Institute.

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TABLE 1. Amount of Total Nitrogen and ^{15}N Label in Earthworms

Soil	Test time, days					
	1		4		28	
	total nitrogen, %	^{15}N, at.%	total nitrogen, %	^{15}N, at.%	total nitrogen, %	^{15}N, at.%
Peat	13,26	0,66	12,05.	0,49	9,23	1,15
Sod-podzolic	13,18	0,41	10,75	0,43	9,94	0,62

TABLE 2. Changes in the Content of Total and Ammonia Nitrogen in Soil after Exposure to Earthworms

Soil	Variants	Nitrogen content			
		total		ammonia	
		%	^{15}N, at.%	mg/100 g of absolutely dry soil	at.%
Peat	Algae	2,141	1,78	12,9	5,4
	Algae + worms	1,684	1,18	26,6	Not det.
Sod-podzolic	Algae	0,242	1,63	2,31	5,3
	Algae + worms	0,221	1,13	3,36	2,7

Used as food in the first experiment was the green alga *Muriella aurantiaca* Vischer, grown on Gromov aqueous medium, in which nitrate nitrogen was replaced by an equivalent amount of $(NH_4)_2SO_4$ with a 40% supplement of ^{15}N; pH of the medium at the beginning of the experiment was 6.4. To remove the physiologic acidity of the salts, 1 mg of $CaCO_3$ was added each week to the medium. After 6 months of cultivation, algae were removed from the medium, washed three times with distilled water, and added to soil placed in clay pots. Two types of soil from the Karelian ASSR were used, peat and sod-podzolic. The amount of algae added to the soil was 24 mg for the first soil and 2.7 mg per gram of absolutely dry soil for the second. The pots were watered according to weight to maintain constant moisture. Earthworms isolated from the same soils were placed in the soil in three series: after 1, 4 and 28 days. Ten earthworms *Lumbricus rubellus* Hoff. were added to the peat and eight worms *Eisenia foetida* Sauv. to the sod-podzolic.

The intestines were removed from the earthworms (found in a pot on the same day) in the first series of tests, and worms extracted from soil were kept on moist filters for a day before removing the intestines in the second and third series. Then the bodies of the worms were dried at 105°C to reach absolute dry weight, ground in a mortar, and total nitrogen and the ^{15}N label were determined in the powder obtained (Table 1).

To establish the role of earthworms in changes in the nitrogen content in soil and in the transformation of algal biomass, control variants were used in the experiment: the same soils with algae but without worms. The content of total nitrogen and the ^{15}N label were determined after 28 days in soils of all variants (Table 2).

In the second experiment, we tested the consumption of ^{15}N-labeled algae by the enchytraeids *Enchytreus albidus* Henle. The yellow-green alga *Chlorocloster pachychlamys* Pasch. and two strains of the green alga *Chlamydomonas reinhardii* Dang. were grown on a medium with $(NH_4)_2SO_4$ with a 10% supplement of the heavy nitrogen isotope. Algae were added to food known to be eaten by enchytraeids, oat cereal, for a week, until a green color appeared. At the end of the experiment, the ^{15}N label in the body of enchytraeids was assayed (Table 3).

Despite the short time required for the passage of food through the intestine of earthworms and the low content of algae in the soil, the nitrogen label was found in the body of worms (Table 1). Usually, accumulation of labeled nitrogen was proportional to exposure time and was the highest in the series of tests where the worms were kept in soil for 28 days

TABLE 3. Content of Labeled Nitrogen ^{15}N in Algae and in enchytraeids Receiving Algae, at. %

Subject of the study	*Chlamydomonas reinhardii* strain 86	*Chlamydomonas reinhardii* strain 162	*Chlorocloster pachychlamys*
Algae	9,6	10,1	10,2
Enchytraeids	0,41	0,48	0,415

and apparently did not pass it through their intestine. During the test, the total nitrogen content in worm tissue decreased, which indicates the beginning of starvation.

Assimilation of labeled nitrogen by algae depends on soil type and apparently on species specificity of worms and algae. In peat soil the worms assimilated substantially more algae than in sod-podzolic, which perhaps is related to the lower adsorption of algae in peat soil and the greater efficacy of digestive enzymes. But far from all nitrogen in algae was assimilated by worms: more than half of the labeled nitrogen remained in soil after worms were removed, as indicated by the comparison of numerical values in variants with and without worms (see Table 2). It is possible that during passage of algae through worm intestine, they are damaged and therefore are not found by microscopy (Atlavinite and Pociene, 1973). But enrichment of soil with labeled nitrogen remains sufficiently high after worms remained in soil for 4 weeks.

Analysis of Table 2 suggests that earthworms actively eliminate not only labeled nitrogen from algae but also nitrogen from the soil, particularly from peat, where the nitrogen content decreased to 22% in the presence of worms. The amount of ammonia nitrogen also increased substantially, i.e., mineralization of nitrogen in organic compounds of soil was 1.5-2-fold more intense than in variants without worms. According to our data (Kozlovskaya, 1976), earthworms transform organic nitrogen into a mobile state. In these experiments, the content of ammonia nitrogen increases substantially, and nitrification processes increase. It is possible that the decrease in the label in the variant with worms is related to the washing out of mineral nitrogen. Denitrification processes are also not excluded. The ^{15}N label was also found in invertebrate bodies in the experiments with enchytraeids (Table 3). Despite the low enrichment of the isotope used and the brevity of the experiment (1 week), the amount of labeled nitrogen in enchytraeid bodies was higher than the natural, and highest in the variant in which enchytraeids were fed *C. reinhardii*.

These experiments demonstrate that algae are not simply swallowed by animals but are digested in the intestinal tract, and their nitrogen is incorporated into invertebrate metabolism. The not far-from-complete assimilation of algae by animals is indicated both by the retention of a significant portion of labeled nitrogen in soil and also by the dissemination of algae: earthworms disseminated unicellular (*Chlorella vulgaris* Beijer., *Botrydiopsis arhiza* Borzi) and filamentous (*Heterothrix exilis* (Klebs) Pascher, *Tribonema minus* Hazen) algae (Kozlovskaya et al., 1975). In our experiments enchytraeids only seeded *Chlorocloster pachychlamys*, and *Chlamydomonas* cells were digested or damaged and not found in enchytraeid excrement. Apparently, selective assimilation of algae may also exist for invertebrates which pass soil or another substrate containing various organic matter through their intestine. Perhaps, for such invertebrates the algae acts as a "vitamin supplement."

Trophic relations are not limited to the interaction between soil oligochaetes and algae. The considerable environment-transforming role of earthworms is known, which can hardly fail to affect the growth of algae. Dozens of tons per hectare of animal excrement enter the soil annually, which is enriched by mobile nutrients, vitamins, and enzymes (Kozlovskaya, 1976), which has a positive effect on plant growth.

In this work we discussed the effect of excrement of earthworms, enchytraeids, and some other invertebrates on the growth of soil algae.

Atlavinite (1975) notes that worms applied to a lawn of algae did not inhibit them with their slime or excrement. On the other hand, there have been references to the toxicity of earthworm excrement for algae (Nekrasova et al., 1976). We checked the effect of the excrement of enchytraeids, springtails, and Diptera larvae on several algal species: *C. rein-*

TABLE 4. Growth of Algae* on Earthworm Coproliths (sod-podzolic floodplain soil, 1978)

Algae species	Control soil	Coproliths
Test 1		
Oscillatoria splendida Grev		+
Phormidium autumnale (Ag.) Gom . . .	++	+
Ph. foveolarum (Mont.) Gom		+
Ph. inundatum Kütz	+	++
Chlamydomonas sp.	+	+
Mesotaenium endlicherianum Näg . . .	+	
Heterothrix bristoliana Pasch . . .		++
Navicula contenta Grun	+	+++
Pinnularia intermedia Lagerst. . . .	+	+++
Hantzschia amphioxys (Ehr.) Grun . .	+	+
Test 2		
Nostoc commune Vauch.	+	
Cylindrospermum sp..		+
Pseudanabaena galeata Böcher		+
Phormidium autumnale (Ag.) Gom . . .	+	
Ph. foveolarum (Mont.) Gom		+
Ph. henningsii Lemm.	+	
Ph. inundatum Kütz	+	++
Ph. sp.		+
Microcoleus vaginatus (Vauch.) Gom .	++	+
M. tenerrimus Gom.	+	
Chlorococcales gen sp.	++	+
Hromidium flaccidum A. Br.		+
Heterothrix exilis (Klebs.) Pasch. .		+
Navicula contenta Grun	+	+
N. sp		+
Stauroneis anceps Ehr.		+
Pinnularia borealis Ehr.		+
P. intermedia Lagerst.		+++
Hantzschia amphioxys (Ehr.) Grun . .	+	+

*The plus signs designate algal abundance.

hardii, *Coccomyxa solorinae*, *Chlorococcum* sp., *Pleurochloris magna* Boey-Pet., and *Chloroclos-ter pachychlamys*. Clumps of excrement of these animals were placed on a lawn of algae, grown on an agar medium. The procedure was also carried out separately with the ground bodies of animals, in which soil was removed from the intestines. The control was dishes with a pure algal culture and dishes to which the peat soil, in which the animals were kept, was added together with excrement and animal bodies. It became obvious that algal growth in dishes with excrement proceeds much more vigrously than in the control, and a narrow band (1-2 mm) of growth inhibition is formed only around the excrement clumps themselves. This inhibition zone is apparently related to the somewhat high concentration of different compounds in the excrement. It is eliminated later and algae "encroach" on the feces.

The ground animal bodies in the soil also exhibited no inhibiting effect on algal growth; on the contrary, the animal bodies stimulated algal growth in all instances, except in the case of *Chlorocloster pachychlamys*.

We studied growth on earthworm coproliths under natural conditions in an area of a flooded meadow. Fresh coproliths and those lying on the soil were collected. Algae were not found on fresh coproliths by direct microscopic examination (including fluorescence microscopy). Coproliths on the soil surface were actively populated by algae (Table 4). The composition of the latter was determined in soil cultures with cover-glasses for growth, i.e., during incubation of coproliths and control soil in light at constant humidity. As is

evident from Table 4, coproliths are populated by approximately the same algae as inhabit the surrounding soil. Algal species diversity, however, is greater on coproliths, and some species grew more vigorously. This applies particularly to diatomaceous algae, which, first of all, are capable of active motion, and secondly, are usually confined to the bare surface of soil and are rarely encountered on litter. It may be suggested that coproliths on the soil surface serve as a site for the multiplication of algae similar to animal excrement, which acts as centers of microbiological activity (Kozlovskaya and Zhdannikova, 1961). In addition, pioneer algal-zoological association are formed here: not only were abundant bacteria and fungi encountered in coprolith cultures, but also diverse invertebrates: colorless flagellates, ciliates, naked and shelled amoebas, and rotifers. The consumption of diatoms by very large amoebas (up to 100 μm) was observed; one amoeba contained 8-10 diatom cells.

Thus, soil oligochaetes actively participate in the control of algal numbers and in the transformation of their biomass. A portion of the algal biomass is assimilated by the worms, another is mineralized and evidently stabilized in soil. The activity of worms in this respect is determined by their species.

Algal numbers in the soil decrease owing to the food-seeking activity of oligochaetes in soil, but worm coproliths promote a new burst of algal growth and an increase in algal species diversity. The burst of algal growth is determined, on the one hand, by the appearance of a free soil surface, and on the other, by the nutritional value of coproliths.

Apparently, algae, as a component of any biogeocenosis, biologically stabilize mobile nutrients, but not for long. The release of nutrients occurs with the participation of oligochaetes and other invertebrates.

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